Regulation of the mutated molecule-mediated ER stress will be another important issue in the future. Knock-in mice that express the mutated cadm1 related to the human CADM1 (H246N) or (Y251S) will provide more insight into the relationship between the ER stress and the pathogenesis of ASD.

Materials and Methods

Protein–protein interaction assay. His-tagged recombinant protein wild-type-CADM1 (48–334 a.a. including three Ig domains) lacking the transmembrane domain were prepared using silkworm cells (Katakura Industries Co., Tokyo, Japan).[35] His-tagged CADM1 (48–334 a.a.) was purified by Ni-column according to the manufacturer's protocol (Qiagen Science, Germantown, MD, USA).

Wild-type or mutated CADM1 in a pcDNA vector was transfected into COS cells using Lipofectamine 2000 (Invitrogen). After incubation for 28 h, the cells were lysed with PBS containing 1% Triton X-100, and then centrifuged at 12 000 r.p.m. for 20 min, COS-cell extracts. His-tagged recombinant Cadm1 (48–334) protein (2 μg protein) was incubated with the extracts (500 μg protein) from COS cells expressing wild-type, H246N-, or Y251S-mutated Cadm1 (48–334) -myc at 4°C overnight. The complexes were isolated from the incubation mixture by binding with the Ni-column and detected by immunoblot analysis using anti-myc (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and evaluated by densitometric analysis. Data from three experiments were scanned and analyzed for quantification with Image J software (National Institutes of Health).

Fluorescence correlation spectroscopy (FCS). TAMRA-labeled recombinant protein (mutated or wild-type Cadm1 (48–334 a.a. including three Ig domains)) lacking the transmembrane domain was prepared using the *in vitro* Pinpoint Fluorescence Labeling Kit 543).[36] TAMRA-labeled and non-labeled recombinant protein were purified using the RTS 100, *E. coli* HY Kit (Roche, Basel, Switzerland). FCS measurements were performed using an MF20 single molecule fluorescence detection system (Olympus, Tokyo, Japan). A helium–neon laser (543 nm) was used for the detection of TAMRA-labeled recombinant protein. TAMRA-labeled recombinant mutated or wild-type Cadm1 (4 nM) was mixed with non-labeled recombinant mutated or wild-type Cadm1 (0–40 nM) and added to the mixture in PBS with 0.05% Tween 20. After the mixtures were incubated at 37°C for 1 h, an aliquot (50 μl) of each sample was transferred to a microplate (24 × 16 wells, Olympus). A standard solution (MF-D543PX, Olympus) was used to derive the optical parameters necessary for a proper measurement. All measurements were carried out in more than duplicate and with 10 scans, each lasting 10 s at room temperature. The obtained data were fitted according to an autocorrelation function embodied in the accompanying software.

Modeling the structure of mutated CADM1. The structure of the Ig2 and Ig3 domains of wild-type and mutant CADM1 were built by SWISS-MODEL[37] using the crystal structure of MuSK (PDB entry: 2IEP) as a template and the amino acid sequence from 163–327 of CADM1. For the modeling of the H246N and Y251S mutants of CADM1, the input sequences were modified corresponding to their mutations.

Transfection of the wild-type and mutated CADM1-myc into neurons. Neurons were isolated from the brains of *cadm1*-deficient mice at embryonic day 16 as described.[38] Neurons were cultured using Neurobasal medium with 2% B27 supplement (Invitrogen, Carlsbad, CA, USA) and L-glutamine (0.5 mM). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 6 days *in vitro* (DIV), neurons were transfected with wild-type or mutated (H246N) or (Y251S) myc-tagged CADM1 using the calcium phosphate method and incubated for 2 DIV.

Immunostaining. For the immunostaining assay for intracellular localization of CADM1 and synaptophysin in the neurons and C2C5 cells, cells were transfected with wild-type and H246N- or Y251S-mutated pcDNA–CADM1 in the presence or absence of 4-PBA (7.5 mM) or rapamycin (10 μg/ml), and fixed in 4% paraformaldehyde, washed with PBS, and then incubated with mouse anti-synaptophysin (Sigma, St Louis, MO, USA), mouse anti-KDEL (Stressgen Biotechnologies Corp., Victoria, BC, Canada), rabbit anti-beclin (Cell Signaling Technology, Beverly, MA, USA), mouse anti-CHOP (Santa Cruz), or chicken anti-SynCAM1 (Cadm1) (MBL, Nagoya, Japan) overnight at 4°C. Alexa Fluor 488- and Alexa Fluor 568-conjugated secondary antibodies against chicken and mouse or rabbit IgG were purchased from Molecular Probes (Eugene, OR, USA). Nuclei were detected by Hoechst 33342 staining (Molecular Probes). The reactivity was viewed using a confocal laser-scanning microscope (CSU-10, Yokogawa, Yokokawa Electric Co., Tokyo, Japan).

Conflict of interest

The authors declare no conflict of interest.

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Impairment of social and emotional behaviors in *Cadm1*-knockout mice

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ABSTRACT

Cell adhesion molecule 1 (CADM1), a member of the immunoglobulin superfamily, mediates synaptic cell adhesion. Missense mutations in the *CADM1* gene have been identified in autism spectrum disorder (ASD) patients. In the present study, we examined emotional behaviors, social behaviors and motor performances in *Cadm1*-knockout (KO) mice. *Cadm1*-KO mice showed increased anxiety-related behavior in open-field and light–dark transition tests. Social behaviors of *Cadm1*-KO mice were impaired in social interaction, resident-intruder and social memory/recognition tests. Furthermore, motor coordination and gait of *Cadm1*-KO mice were impaired in rotarod and footprint tests. Our study demonstrates that CADM1 plays roles in regulating emotional behaviors, social behaviors and motor performances, and that CADM1 has important implications for psychiatric disorders with disruptions in social behavior, such as autism.

1. Introduction

CADM1 (previously known as TSLC1, IGSF4, SgIGSF, SynCAM1, Necl2, RA175), a member of the immunoglobulin (Ig) superfamily, is a synaptic cell adhesion molecule [1–4]. CADM1 contains three extracellular Ig-like domains followed by a single transmembrane region and a short cytosolic sequence that interacts with PDZ (post-synaptic density-95 (PSD-95)/Discs large/zona occludens-1) domains of synaptic scaffolding molecules [1]. CADM1 is required for synapse formation and maturation of presynaptic terminals [1–4].

Recently, we identified two missense mutations, C739A (H246N) and A755C (Y251S), in the *CADM1* gene of male Caucasian autism spectrum disorder (ASD) patients and their family members [5]. Both mutations are located in the third Ig domain of CADM1, which is essential for cell adhesive *trans*-interaction [1]. The mutated CADM1 exhibited defective trafficking to the cell surface and more susceptibility to the cleavage and/or degradation. From these results, we suppose that phenotypes in ASD patients might partly correlate with impaired synaptogenesis induced by these mutations.

In the present study, to assess the roles of endogenous CADM1 in behavioral functions, we examined emotional behaviors, social behaviors and motor performances in *Cadm1*-KO mice. We used *Cadm1*-KO mice on a C57BL/6J background.

2. Materials and methods

2.1. Animals

Cadm1-KO mice [6], heterozygous mice and wild-type mice were obtained from heterozygous intercrosses on the C57BL/6J background for 10 generations. All behavioral studies were performed between 13:00 and 17:00 using *Cadm1*-KO, heterozygous and their littermate wild-type male mice. Animals had free access to food and water under the condition of 12-h light–dark cycle (22 ± 2 °C, 40–70% humidity, light on between 7:30 and 19:30). Experimental protocols used throughout the study were approved by an institutional committee and were in accord with Japanese legislation concerning animal experiments.

2.2. Behavioral studies

2.2.1. Open-field test

To assess spontaneous locomotor activity and anxiety-related behavior, 10–15-week-old mice were placed in a corner of an

Abbreviations: CADM1, cell adhesion molecule 1; ASD, autism spectrum disorder; KO, knockout; Ig, immunoglobulin; ANOVA, analysis of variance; ER stress, endoplasmic reticulum stress.

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open-field apparatus (60 × 60 × 40 cm; O'Hara and Co., Tokyo, Japan) with an illuminated (60 lux) chamber. The distance traveled and time spent in the center area were recorded over a 10-min period.

2.2.2. Light–dark transition test

Anxiety-related behavior was tested using the light–dark transition test. The apparatus consisted of a cage (40 × 40 × 30 cm) divided into two equal chambers by a black partition containing a small opening (O'Hara and Co., Tokyo, Japan). One chamber was made of white plastic and was brightly illuminated (100 lux), whereas the other chamber was black and dark. Ten to fifteen-week-old mice were placed in the dark chamber and allowed to move freely between the two chambers for 5 min. The time spent in each chamber and the total distance traveled was recorded.

2.2.3. Elevated plus-maze test

The apparatus consisted of two open (25 × 5 cm) and two enclosed arms of the same size with 15-cm-high transparent walls (60 lux, O'Hara and Co., Tokyo, Japan). The frequency of entry into open arms, the time spent in open arms and locomotion activity were measured, as described previously [7]. Eleven to sixteen-week-old mice were used.

2.2.4. Social interaction test

Social behavior between two mice in a familiar environment was tested with a system that automatically analyzes behavior in home cages [7]. The system consisted of a cage (29 × 18 × 12 cm) and a filtered cage top containing an infrared video camera and infrared light emitting diodes. Two genetically identical mice (20–25 weeks old) that had been housed separately were placed together in a home cage and their behavior was video-monitored over 2 days. Images from each cage were captured at a rate of one frame per second. Social interaction was measured by counting the number of particles in each frame; two particles indicated that the mice were not in contact with each other and one particle indicated contact between the two mice.

2.2.5. Hand-scored social behaviors in social interaction test

Behaviors were recorded on digital video and social behaviors of two mice (23–31 weeks old) were observed during the first 5 min following placement of the two mice into the test cage. Behaviors were categorized into the following nine indices; non-social exploratory behaviors, approaching, following, anogenital sniffing, nose-to-nose sniffing, crawling over and under, grooming each other, sleeping together, and fighting.

2.2.6. Resident-intruder test

Resident males (51–56 weeks old) were individually housed for 4 weeks before testing. Male C57BL/6J mice at the age of 4–5-week-old housed in groups were used as intruders. Two tests of 5-min duration were performed at a 5-min interval. New intruder mice were used in each test. Latency to attack the intruder, total duration of attack, and total frequencies of attack were measured.

2.2.7. Social memory/recognition test

Social investigating behavior was measured by social memory/recognition test. Eleven to seventeen-week-old male mice were transferred to an individual cage. Stimulus mice were ovariectomized more than one week prior under Avertin anesthesia [8,9]. A transparent acrylate cylinder (7 cm diameter) with several small holes in its lower portion was placed in the center of the cage. Two hours after transferring the test mice, the ovariectomized stimulus mouse was introduced into the cylinder for a 5-min confrontation. At the end of the 5-min trial, the stimulus animal was removed. This was repeated with the same stimulus mouse for three trials with 15-min intervals between them. In a fourth trial, a different ovariectomized stimulus mouse was introduced to the resident male mouse. The time spent investigating the intruder was measured in each trial. Ratios of the time spent by each subject on olfactory investigation during the fourth trial (exposure to a novel mouse) as compared with the time spent during the third trial (exposure to the familiar mouse) was calculated as an index of social memory/recognition [10].

2.2.8. Rotarod test

Motor coordination and motor learning were tested using the rotarod test. Mice (13–19 weeks old) were placed on a rotating drum (3 cm diameter) (O'Hara and Co., Tokyo, Japan). The speed of the rotarod accelerated from 4 to 40 rotations over a 5-min period. How long it took for the mice to fall off the rotarod was recorded. Three trials were performed per day for two days.

2.2.9. Footprint test

Mice (14–22 weeks old) were trained three times to walk along a 50-cm-long, 10-cm-wide corridor (with 30-cm-high walls) toward a dark compartment. Before the fourth trial, their hind paws were dipped in India ink and were then allowed to walk on paper strips. Stride length, interstep and hindpaw base width were measured from three steps.

2.2.10. Buried food pellet test

The food was buried under 5 cm of bedding in one corner of the cage. The mouse, which had been fasted for 18 h, was placed in the center of the cage, and the time taken to find the food was recorded for wild-type and *Cadm1*-KO mice (21–28 weeks old).

2.2.11. Grip strength test

Grip strength was measured by letting forehands of 14–20-week-old mice grip mesh connected to a spring balance and pulling the bodies until the mice released the mesh (O'Hara and Co., Tokyo).

2.2.12. Tail immersion and hot plate tests

Those tests were used to examine pain sensitivity. At 14–19 weeks of age, the tail of each mouse was immersed 2 cm into water at 48 °C and the latency time of a rapid tail flick was measured five separate times on each animal over a 15-min period, allowing a 3-min recovery period between each trial. For the hot plate test, mice were placed on a 54 °C hot plate and latency of the first paw response (a foot shake or a paw lick) was recorded.

2.2.13. Tail suspension test

The tail suspension test was used to assess depression-related behavior. Mice (14–19 weeks old) were suspended by the tail for 6 min and cumulative immobility time was recorded.

2.3. Image analysis and statistics

The applications used for the behavioral studies were Image OP, Image LD, Image EP, Image FZ (O'Hara and Co.), which were produced on the basis of the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/), and Image J program (http://rsb.info.nih.gov/ij/). Data, presented as means ± SEM, were analyzed using one-way analysis of variance (ANOVA) or repeated measures ANOVA followed by a Dunnett's *t*-test for multiple comparisons. Single comparisons were made using a Mann–Whitney U test or Wilcoxon signed-rank test.

3. Results

We tested the mice for anxiety-related behaviors using the open field, light–dark transition, and elevated plus-maze tests. The *Cadm1*-KO mice showed reduced center time in the open-field test (Fig. 1A) and less time in the light box of the light–dark test (Fig. 1B). Because the total distance in the open field or light–dark test did not show a significant genotype-dependent difference, the lessened time in the center or in the light box was not attributable to locomotor impairment of *Cadm1*-KO mice. These two test results suggested enhanced anxiety-like behavior in *Cadm1*-KO mice. However, the *Cadm1*-KO mice did not show significant differences in stay time in or entries into open arms of the elevated plus-maze test (Fig. 1C). The increased anxiety-like behaviors in *Cadm1*-KO mice may only be manifested under certain situations.

We also evaluated social behaviors of the mice. First, direct contact between pairs of genotypically identical *Cadm1*-KO or wild-type mice was analyzed for 24 h. In the light period, wild-type mice spent majority of time staying still in contact with each other, while the *Cadm1*-KO mice showed higher locomotion and spent less time in contact with each other (Fig. 2A). Second, we also hand scored the actual social interaction behaviors during the first 5-min period after placement of two mice into a new cage. The *Cadm1*-KO mice spent less time exploring the cage and more time following the other mice as compared to wild-type mice (Fig. 2B). The time spent fighting was also longer for the *Cadm1*-KO mice. The social interaction behaviors of heterozygous mice were not significantly different as compared to wild-type mice (Fig. 2B). In a resident-intruder test, the *Cadm1*-KO mice showed shorter attack latency, longer attack duration, and higher attack frequency (Fig. 2C), suggesting higher aggressive behavior in these mice.

Furthermore, we measured social investigating behavior in a social memory/recognition test, in which the same ovariectomized female mouse was repeatedly used for exposure. The wild-type mice exhibited a characteristic decrease in the time spent investigating the female upon repeat exposures (85.6 ± 6.2 s for the first exposure, 61.8 ± 7.2 s for the third exposures. $P < 0.05$), with a full recovery following the introduction of a new female (91.0 ± 10.2 s). The *Cadm1*-KO mice exhibited no decrease in investigation time of the same female mouse upon repeat exposures (89.8 ± 8.1 s for the first exposure, 85.1 ± 11.1 s for the third exposures. $P > 0.05$). When we presented the *Cadm1*-KO males with a new female, they spent a similar time investigating the new female (79.9 ± 12.1 s) as compared to that investigating the familiar animal. The ratio of time spent investigating a novel mouse (fourth trial) compared to a familiar animal (third trial) was significantly less in the *Cadm1*-KO mice than for wild-type mice (Fig. 2D). Thus, the *Cadm1*-KO mice showed impairment in forming familiarity, a core symptom of ASD patients.

The *Cadm1*-KO mice also had deficits in motor learning using the rotarod test (Fig. 3A). The time to falling off the rotating cylinder was longer after repeated training for wild-type mice, but not for the *Cadm1*-KO mice. This result may be related to the clumsiness in gross motor function that people with ASD often exhibit.

We also examined gait pattern in a foot print test. Neither stride length nor interstep were significantly different between wild-type

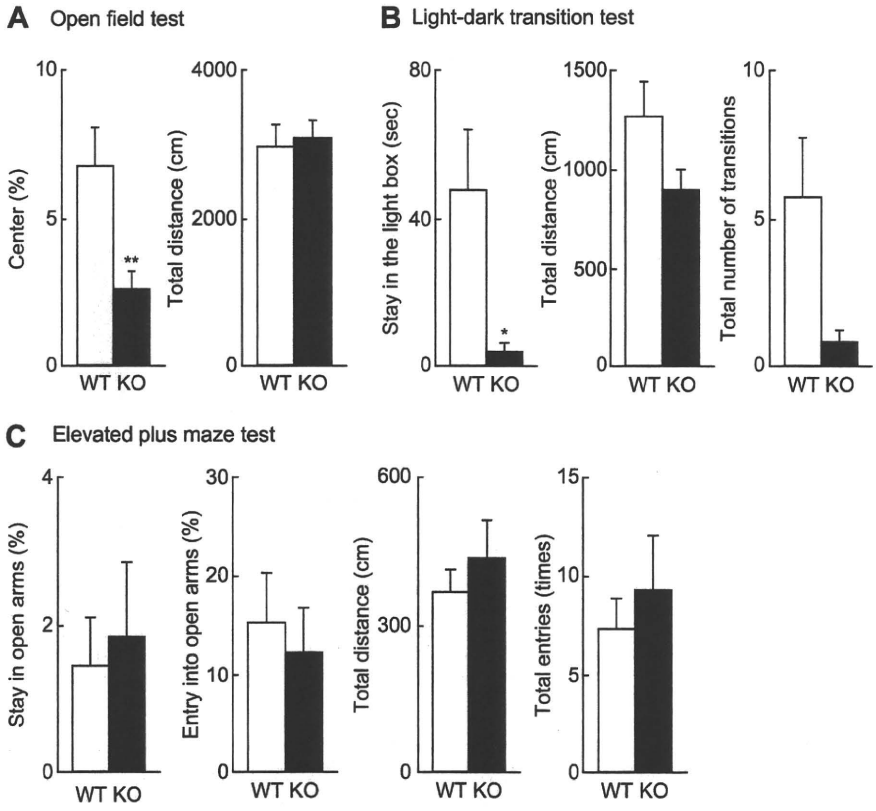

Fig. 1. Anxiety-related behaviors of *Cadm1*-KO mice. (A) The percentage of time spent in the center area of an open field cage and total distance traveled during the open-field test. *Cadm1*-KO mice ($n = 11$) spent less time in the center of the open field cage than wild-type mice ($n = 12$), but locomotor activity is similar between KO and wild-type mice. (B) The time spent in the lighted side of a cage during the light–dark transition test and total distance traveled. *Cadm1*-KO mice ($n = 11$) spent less time in the lighted side as compared to wild-type mice ($n = 12$). (C) The percentage of time spent in the open arms, that of the number of entries into the open arms, total distance of moving, and the total number of entries during the elevated plus-maze test. There were no significant differences in these parameters between wild-type ($n = 12$) and *Cadm1*-KO ($n = 10$) mice. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to wild-type mice.

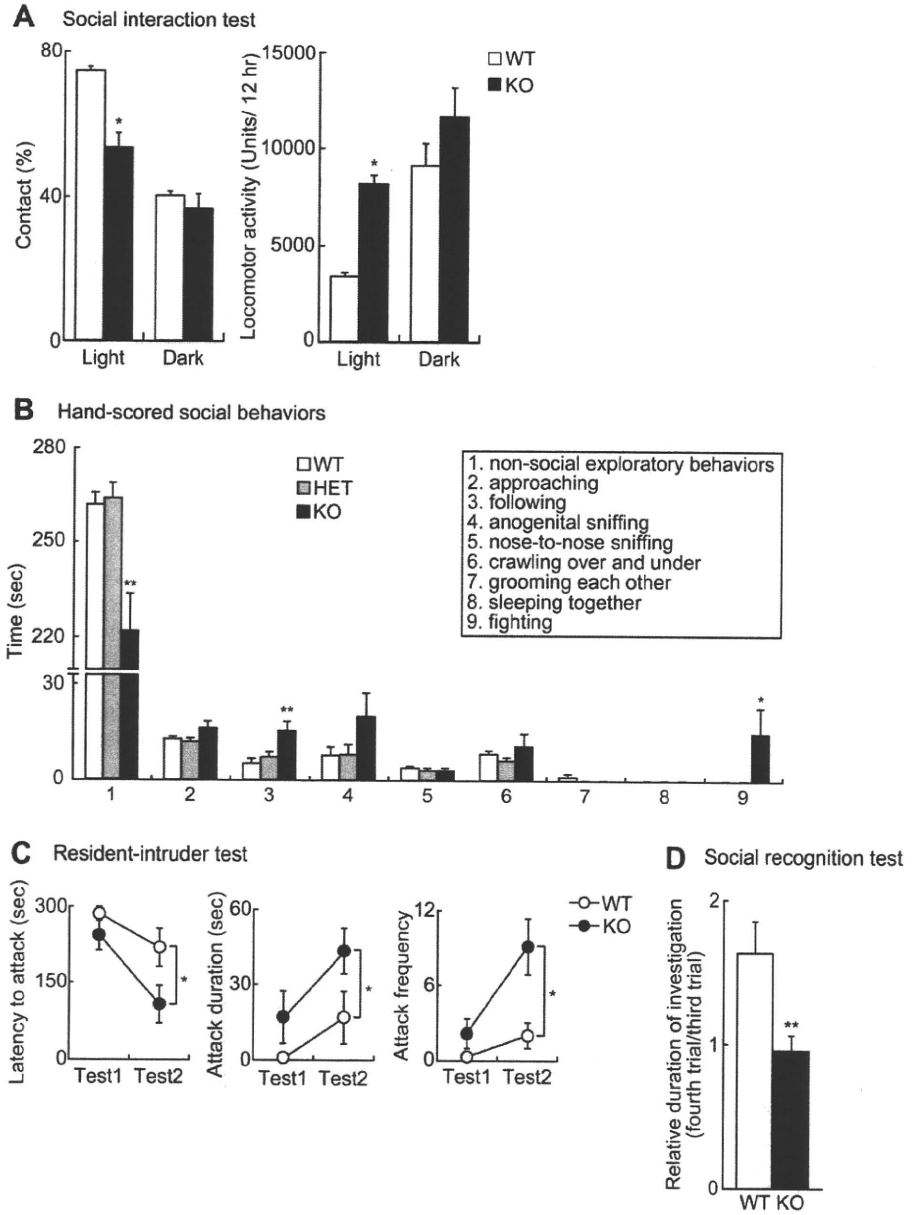

Fig. 2. Social behaviors of *Cadm1*-KO mice. (A, B) The percentage of time spent in contact and locomotor activity (A), and social behaviors (B) in the social interaction test. *Cadm1*-KO mice spent less time in contact with each other in the light period. In the light period, exploratory activity was higher in *Cadm1*-KO mice ($n = 4$ pairs) than in wild-type mice ($n = 4$ pairs). *Cadm1*-KO mice ($n = 7$ pairs) spent less time for exploring the cage and more for following the other mice and for fighting as compared to wild-type mice (n = 8 pairs) or heterozygous mice ($n = 8$ pairs). The one-way ANOVA F and P values were as follows. 9.00 and 0.0016 for non-social exploratory behaviors, 2.28 and 0.13 for approaching, 6.62 and 0.0062 for following, 2.39 and 0.12 for anogenital sniffing, 0.50 and 0.61 for nose-to-nose sniffing, 0.86 and 0.44 for crawling over and under, 0.93 and 0.41 for grooming each other, and 3.91 and 0.037 for fighting. (C) Latency to attack the intruder, total duration of attack, and total frequencies of attack in the resident-intruder test. *Cadm1*-KO mice ($n = 7$) showed shorter attack latency, longer attack duration and higher attack frequency than wild-type mice ($n = 9$). The ANOVA F and P values were as follows. Latency to attack: genotypes: $F = 5.01$, $P = 0.042$, trials: $F = 13.2$, $P = 0.0027$, interaction between genotypes and trials: $F = 1.71$, $P = 0.21$. Attack duration; genotypes: $F = 5.01$, $P = 0.042$, trials: $F = 8.13$, $P = 0.013$, interaction between genotypes and trials: $F = 0.55$, $P = 0.47$. Attack frequency; genotypes: $F = 8.60$, $P = 0.011$, trials: $F = 20.13$, $P = 0.0005$, interaction between genotypes and trials: $F = 8.81$, $P = 0.010$. (D) Relative duration of investigation in the social memory/recognition test. Ratio of time spent for investigating the familiar animal (the third trial) as compared to that for a novel animal (fourth trial) were less in *Cadm1*-KO mice ($n = 9$) than in wild-type mice ($n = 11$), indicating that wild-type but not *Cadm1*-KO mice discriminated between familiar and novel mice. $^*P < 0.05$, $^{**}P < 0.01$ as compared to wild-type mice.

and *Cadm1*-KO mice (Fig. 3B). Only hindpaw base width was significantly increased in the *Cadm1*-KO mice compared to wild-type mice (Fig. 3B), suggesting a wide-based gait, a sign of cerebellar dysfunction.

On the other hand, we identified no significant changes in sensory functions between wild-type and *Cadm1*-KO mice. We found no significant differences between wild-type and KO mice for olfactory function (buried food pellet test) (Fig. 4A), visual function (visual placing test) (data not shown), auditory function (startle response test) (data not shown).

We also found no significant differences in muscle strength between *Cadm1*-KO and wild-type mice as measured by grip strength (Fig. 4B), or in pain sensitivity as measured by tail immersion and hot plate tests (Fig. 4C and D). There was also no significant difference in immobility time in a tail suspension test, suggesting no differences in depression-like behavior in *Cadm1*-KO mice (Fig. 4E).

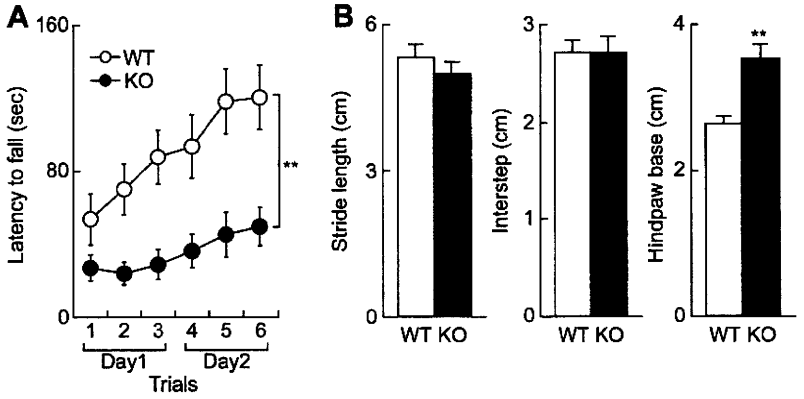

Fig. 3. Rotarod test and footprint test of *Cadm1*-KO mice. (A) The latency at which mice fell off a rotarod during multiple trials. *Cadm1*-KO mice ($n = 10$) showed no improvement in motor coordination, while wild-type mice ($n = 12$) showed improvement after repeated training. The ANOVA F and P values were as follows. genotypes: $F = 10.92$, $P = 0.0035$, trials: $F = 11.97$, $P < 0.0001$, interaction between genotypes and trials: $F = 2.57$, $P = 0.031$. (B) *Cadm1*-KO mice ($n = 11$) showed significantly longer hindpaw bases as compared to wild-type mice ($n = 16$). $^{**}P < 0.01$ as compared to wild-type mice.

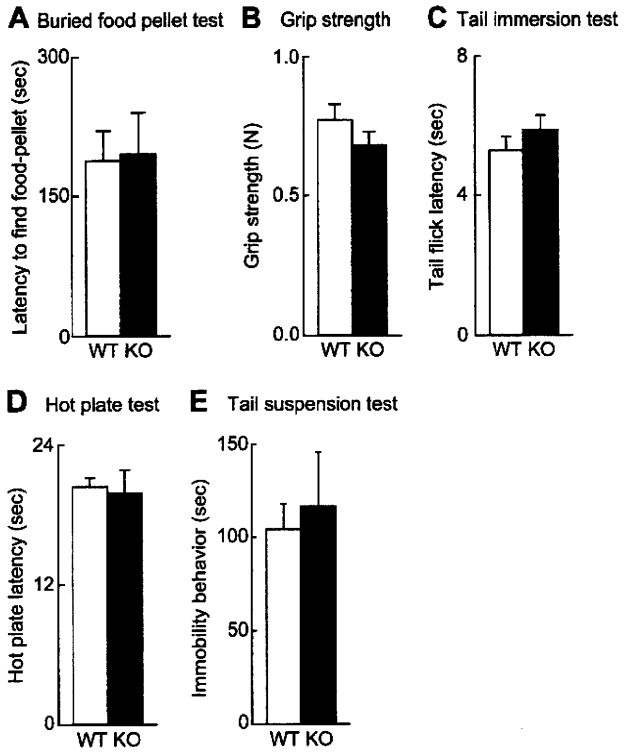

Fig. 4. Olfactory function test (A), grip strength test (B), pain sensitivity test (C and D) and depression-like behavior test (E) of *Cadm1*-KO mice. (A) There was no significant change in latency to find a buried food pellet between wild-type ($n = 16$) and *Cadm1*-KO ($n = 15$) mice. (B) Grip strength was not significantly different between *Cadm1*-KO ($n = 10$) and wild-type ($n = 12$) mice. (C, D) Latencies of tail flick in a tail immersion test (C) and of paw responses in a hot plate test (D) were not significantly different between *Cadm1*-KO ($n = 9$ or 10) and wild-type ($n = 12$) mice. (E) Cumulative time spent for immobility, depression-related behavior, in a tail suspension test was not significantly different between *Cadm1*-KO ($n = 9$) and wild-type ($n = 11$) mice.

4. Discussion

In the present study, *Cadm1*-KO mice exhibited increased anxiety-related behaviors, impaired social behaviors and impaired motor function, while they showed no significant changes in sensory functions. Similar psychiatric or psychosocial symptoms are often observed in people with ASD. Missense mutations in the *CADM1* gene have been identified in ASD patients [5]. These results are consistent with an idea that deficiency in CADM1 functions is involved in pathogenesis of ASD.

The open-field and light–dark transition tests indicated increased anxiety-related behaviors, but the elevated plus-maze test did not show any genotype-dependent difference; it may be that increased anxiety-like behaviors in *Cadm1*-KO mice manifest only under certain conditions. The social interaction and social memory/recognition tests indicated impaired social behaviors in *Cadm1*-KO mice. Furthermore, aggressive behaviors were observed in the *Cadm1*-KO mice. Results with the resident-intruder test suggested that *Cadm1*-KO mice have qualitative impairment in social interaction. Impaired social interaction is one of the core symptoms of ASD. Additional psychiatric or psychosocial symptoms that are often observed in people with ASD are anxiety and aggression [11]: anxiety has been reported to be related to social deficits and loneliness in people with ASD, and aggression is identified in the ASD-BPA (Autism Spectrum Disorders-Behavior Problems for Adults) as an associated symptom of ASD, although the precise nature of the relationship remains unclear [12].

The *Cadm1*-KO mice showed deficits in motor learning, based on findings using the rotarod test, and also showed the increased hindpaw base width, suggesting possible cerebellar dysfunction in *Cadm1*-KO mice. These phenotypes may be an indication of what underlies the problems with gross motor function that people with ASD often exhibit [13]. CADM1 is expressed in the Purkinje cells in the developing brain [14]. It is of interest that impaired development of Purkinje cells has been observed at autopsy in the brains of people with ASD [15]. Cerebellar impairment may be one of the major loci of ASD associated with communication impairment, an interesting question to address in future studies. On the other hand, we found no significant difference in immobility time in a tail suspension test (i.e., no depression-like behavior) and pain reactions in tail immersion or hot plate tests in *Cadm1*-KO mice. While depression and changes in pain reactions can be associated with patients with ASD, association of these comorbid symptoms depends on clinical situations of patients [16,17].

CADM1 is required for synapse formation and maturation of presynaptic terminals [1–4]. Thus the abnormal behavioral and motor phenotypes observed in *Cadm1*-KO mice might be caused by abnormal synaptogenesis due to loss-of function of CADM1. On the other hand, cells expressing the mutated CADM1, which was identified in some people with ASD, show defective trafficking of the mutated protein to the cell surface and its increased intracel-

lular accumulation [5]. Intracellular accumulation of the mutated molecules induces endoplasmic reticulum (ER) stress, which is a possible pathogenesis of ASD [18,19]. Thus both a loss-of function and a gain of function due to mutation of CADM1 may be involved in the pathogenesis of ASD.

Our study provides direct evidence that CADM1 is involved in the development of psychiatric disorders with disruptions in social behavior. *Cadm1*-KO mice could be used as a tool for a therapeutic model for psychiatric disorders with disruptions in social behavior such as ASD. Further study on the assembly of CADM1 with scaffold proteins and receptors on the postsynaptic membrane and its impairment in the *Cadm1* mutation knock-in mice will provide further insights into the pathogenesis of ASD.

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